July 24, 1951.

Dr. C. B. van Niel,
Hopkins Marine Station,
Pacific Grove, California.

Dear Kees-

Esther and I have been working lately a bit on the old and thorny problem of "pre-adaptation"- whether phages and drugs and so forth simply select pre-existent spontaneous mutants, or whether they may directly induce resistance. Our convictions have been stronger than the evidence in favor of the first hypothesis as accounting for most if not all examples of inherited adaptive changes.

In our approach to the problem, we have been using what we shall call a "replica plating method". Colonies or films of growth on the surface of an initial agar plate are transferred by means of a sheet of fabric (velvet or velveteen) to a series of fresh plates, to which various things may be added. The velvet may be thought of as a "rubber-stamping" device, or better as a large assembly of small inoculating needles held in fixed relative position. At any rate, this method has worked unexpectedly well in preparing replicas to different media. It was initially devised to economize on the work needed in the isolation and classification of mutants and recombinants, and has been used in that connection for scoring auxotrophs, fermentative differences (on EMB agar), and resistance to drugs or phages. My main question to you is whether such a procedure has been described before. I found it rather difficult to look into the literature for it, for lack of a suitable heading. The closest I could find was the "Klatschpraeparat", but as far as I could tell this was exklusively an impression-smear method for microscopic examination. If you have any suggestions on this point? I should be grateful to have them.

The replicas are used in two ways for the present problem. For example, films of growth on plain agar can be proven to contain <u>clones</u> of phage-resistants, for serial replicas to phage-coated agar show a large proportion of congruent, superimposable resistant colonies. But beyond this, the phage mutants can be selected indirectly by taking fresh inocula from the sites on the first plate corresponding to the congruences on sister, phage-coated plates. With a few successive enrichments in this way, resistant mutants have been isolated from cells never directly exposed to the selective agent. We are in the process of similar experiments with streptomycin resistance, which is more difficult owing to the extremely low mutation rate.

Yours sincerely,

Joshua Lederberg